The Effects of Glyphosate on Growth, Metal Concentration and Distribution in Transgenic Soybean

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ABSTRACT

Roundup Ready® transgenic soybeans have bacterial genes incorporated into their genome that provide an alternative biochemical pathway for the production of essential amino acids allowing the transgenic soybean to resist the effects of glyphosate(Roundup®). In the nontransgenic plant this biochemical pathway is blocked by glyphosate, ultimately resulting in the death of the plant. The complete effects of the genetic modification are unknown, particularly with respect to effects on metal homeostasis. This experiment measured plant growth and metal concentration and distribution in glyphosate-treated transgenic soybean (*Glycine max*) and nontransgenic or conventionally bred soybean (cv. *Dwight)* as a way to investigate some effects of this genetic modification. Nontransgenic (which we refer to as wild type or WT) and transgenic (which we refer to as genetically modified or GMO) soybean were grown in greenhouse conditions. There were no statistically significant differences in the plant height or biomass of GMO and WT soybean, with or without applied glyphosate. Metal concentration and intracellular metal distribution were analyzed by synchrotron X-ray fluorescence (sXRF) at beamline 13-ID-E. Samples of stem and root were prepared for analysis by cryogenic embedding in OCT and sectioned by hand-held microtome. Sections were preserved by dessication. Leaves were analyzed in vivo directly sampled off living plants at the time of analysis. All samples were mounted on Kapton film for sXRF analysis. Although statistically significant reduced levels of calcium were found in leaf vascular and mesophyll tissue in both WT and GMO soybean treated with glyphosate, the leaves of WT soybean sprayed with glyphosate display significantly larger overall decrease in Ca abundance in leaf mesophyll tissue. It is hypothesized that glyphosate may affect the homeostasis of calcium within the soybean leaf and that the genetic modification that confers glyphosate resistance may partially provide an alternative pathway for Ca incorporation not seen in wild type.

INTRODUCTION

In the 1990's GMO glyphosate resistant soybeans, commercially called Roundup Ready®, were developed. This genetic modification gave the soybean resistance to the effects of glyphosate by providing an alternative biochemical pathway for production of vital amino acids. In farming, the benefits of glyphosate resistant soybeans include less tilling and fewer applications of herbicide by killing competitive weeds without affecting the soybean crop. The majority of soybean varieties commercially grown are glyphosate resistant. There is concern regarding the nutritional value of the transgenic soybean seed crop. The literature shows conflicting data in regards to the effect glyphosate has on the photosynthetic capacity and the intracellular homeostasis of metals in the transgenic soybean (Duke et al., 2012).

Figure 1: Soybeans; pot 1-WT without glyphosate , pot 2-WT glyphosate treated, pot 3-GMO without glyphosate, pot 4-GMO glyphosate treated

METHODS

Plant Growth

GMO and WT soybean plants were grown in greenhouse conditions (Figure 1). Seeds were planted in equal parts perlite, sand and soil. Eight of sixteen transgenic and nontransgenic plants were sprayed with a 1.42% glyphosate solution. Foliar application of glyphosate occurred at the emergence of the fourth trifoliate leaf. Height was determined from soil base to stem top. Plant biomass was determined after drying all plant matter in the microwave; progressively drying with short bursts of energy until mass did not change. Both plant height and total biomass was measured 1 week after glyphosate application.

Figure 2: Students embed samples in OCT in preparation for cryogenic microtoming.

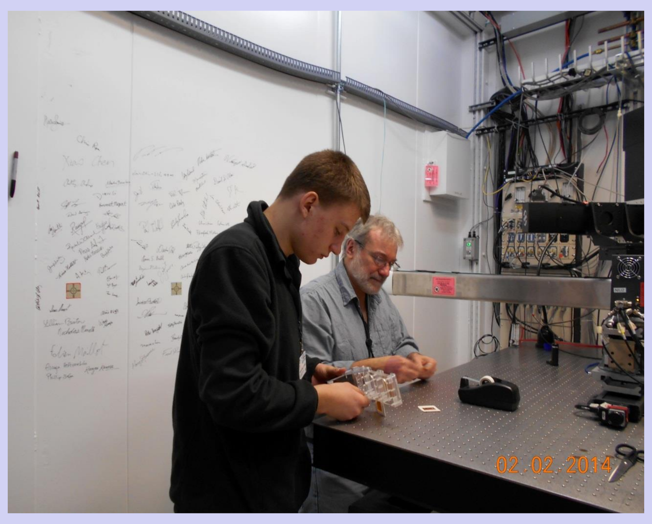


Figure 3: Student and Dr. Lanzirotti load soybean samples into 13-ID-E sample holder for analysis.

Sample Preparation

Samples of stem and root were prepared for analysis by sXRF by embedding plant material in OCT (Optimal Cutting Temperature) compound, freezing in liquid nitrogen and sectioning with a hand-held microtome. Microtomed sections were of multiple cell layers. Progression when using hand-held microtome for sectioning was not uniform, therefore, there was variability in sample thickness. Samples were preserved in a dessicator. Live plants were brought to the APS for in vivo sampling of leaves. All samples were placed on Kapton film.

Elemental Analysis

Samples were analyzed at the **GSECARS 13-ID-E** X-ray microprobe beamline at the Advanced Photon Source, Argonne National Laboratory. Run conditions were as follows:

- Incident beam energy of 12 keV, tuned using Si(111) monochromator
- 1 µm focused beam was used for analyses, focused using 200 mm long, Rh-coated , Kirkpatrick-Baez focusing mirrors.
- X-ray fluorescence collected using 4-element Vortex silicon drift detector.
- Whole stem and root and an approximate 2mm square area of leaf was compositionally mapped and then a summed Energy Dispersive Spectra for this area was used for elemental evaluation (Figure 7).
- The fluorescent counts are considered to be directly proportional to elemental concentration in samples. Samples are assumed to be approximately equal in thickness.

RESULTS

Plant Growth

WT and GMO soybeans with and without applied glyphosate displayed no significant difference in total plant height or biomass. The rate of growth and total height of all soybeans were very similar. T-tests determined no statistical difference in growth measures (Figures 4 and 5). Both height and biomass give a measure of photosynthetic output of the soybean plants.

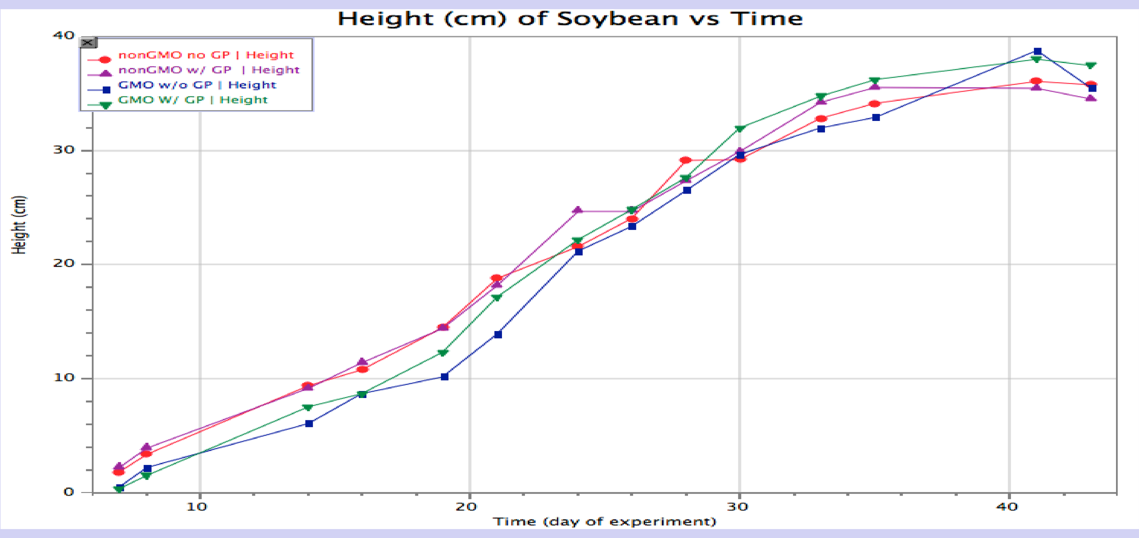


Figure 4: Soybean plant height measured over 6 weeks.

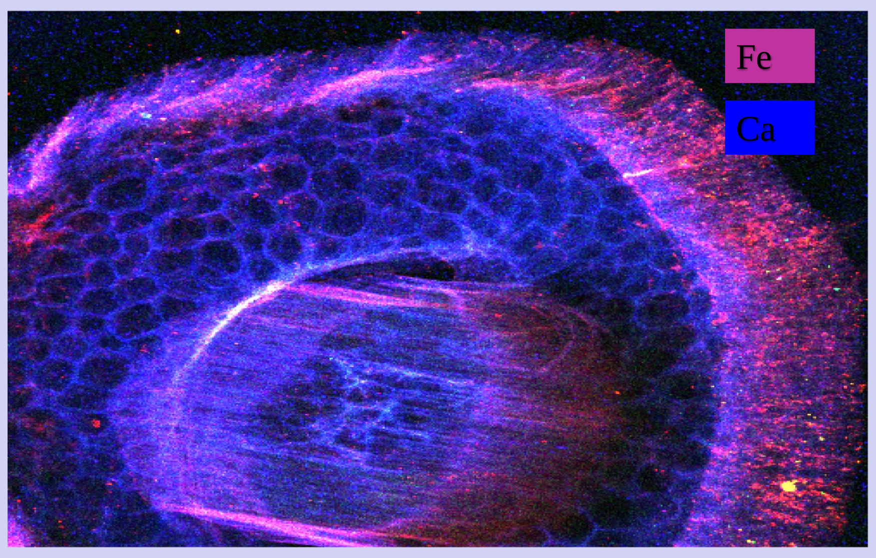
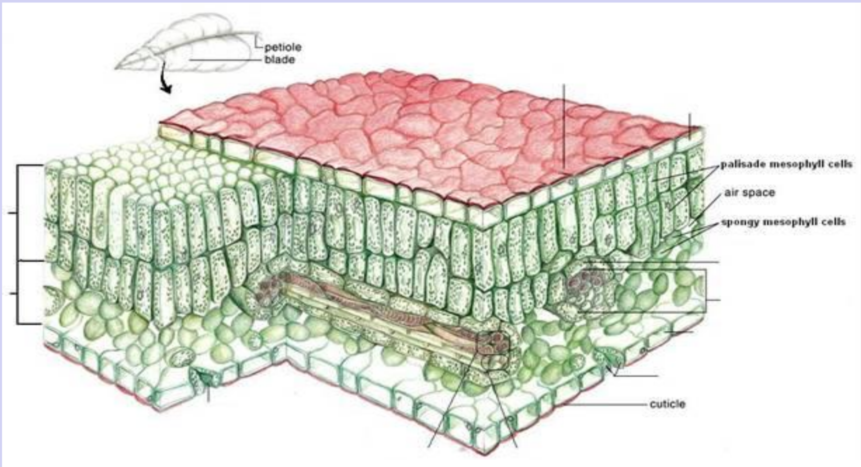


Figure 6: Elemental distribution in transgenic soybean root (no glyphosate applied) collected by sXRF imaging. The central area of map is vascular tissue, surrounded by storage tissue which is encircled by epidermal tissue.



"Leaf Internal Structure." ClarendonCollege.edu. 25 April 2014 <http://www.clarendoncollege.edu/programs/NatSci/Biology/Botany/online%20lecture%20outlines/leaves.html>

Figure 8: Example leaf anatomy showing placement of mesophyll

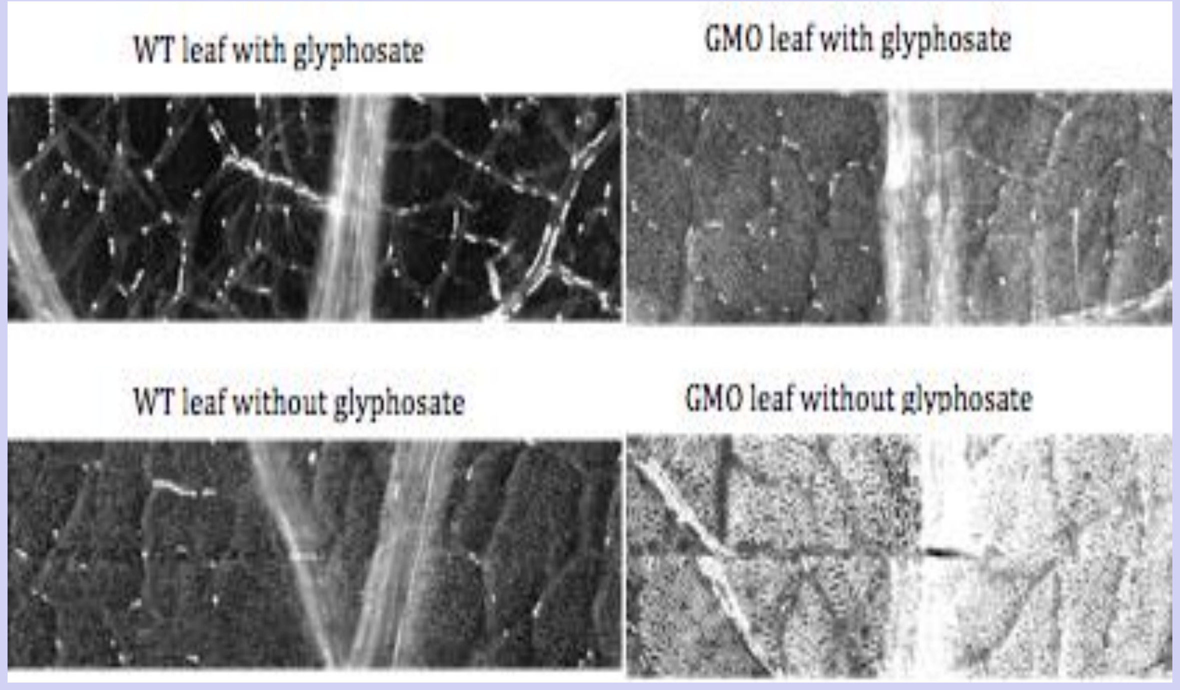


Figure 9: Deposition of calcium in soybean leaf. White scaled to greater concentration. Localized areas of white showing increased levels of calcium.

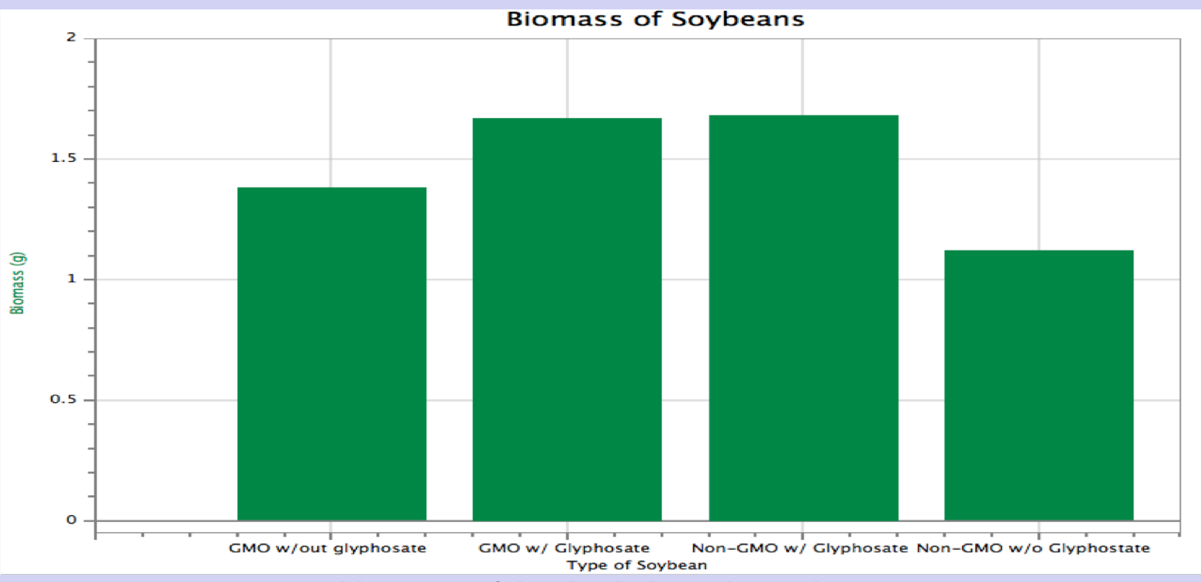


Figure 5: Total biomass of dried, whole soybean plants.

Elemental Analysis

The root and stem elemental analysis of all soybean types by sXRF displayed no significant variations in concentration and distribution.

Leaf mesophyll (the nonveinal tissue) of both the WT and GMO soybean treated with glyphosate had reduced calcium concentrations when compared to soybeans not treated with glyphosate (Figure 10). There was a 30% reduction in calcium counts in GMO leaf mesophyll and 70% calcium count reduction in the WT leaf mesophyll when glyphosate was applied. These differences in mesophyll calcium concentration were statistically significant, $p<0.05$. Deposition of calcium varied in the soybean leaf. In all leaf types there were multiple small isolated areas of highly concentrated calcium that are hypothesized to be sequestered calcium in the form of calcium oxalate (Figure 9).

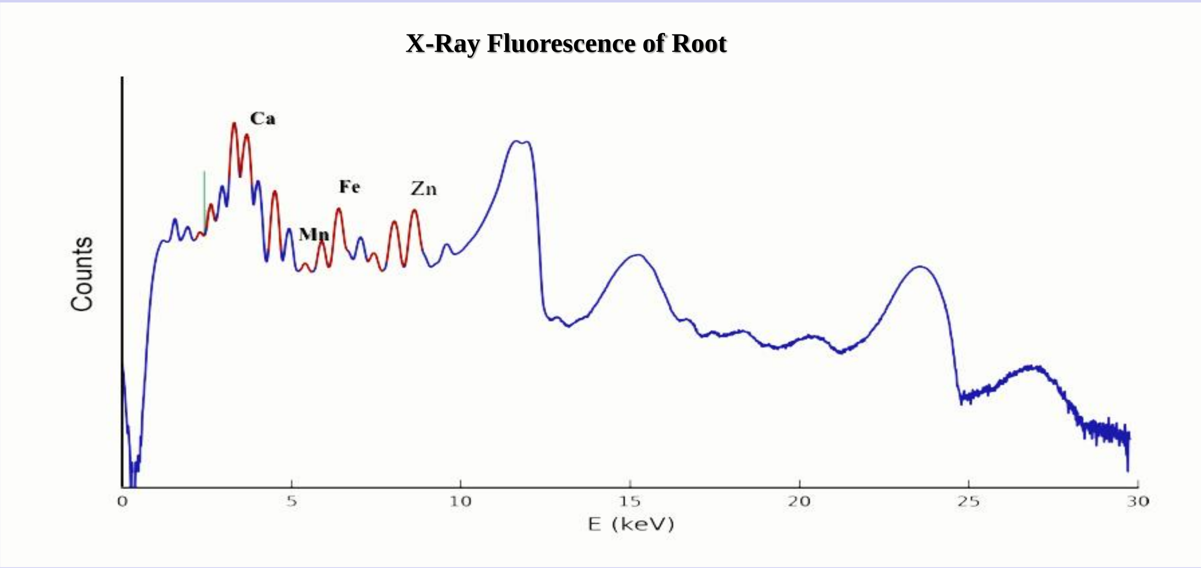


Figure 7: Example energy dispersive spectra of root

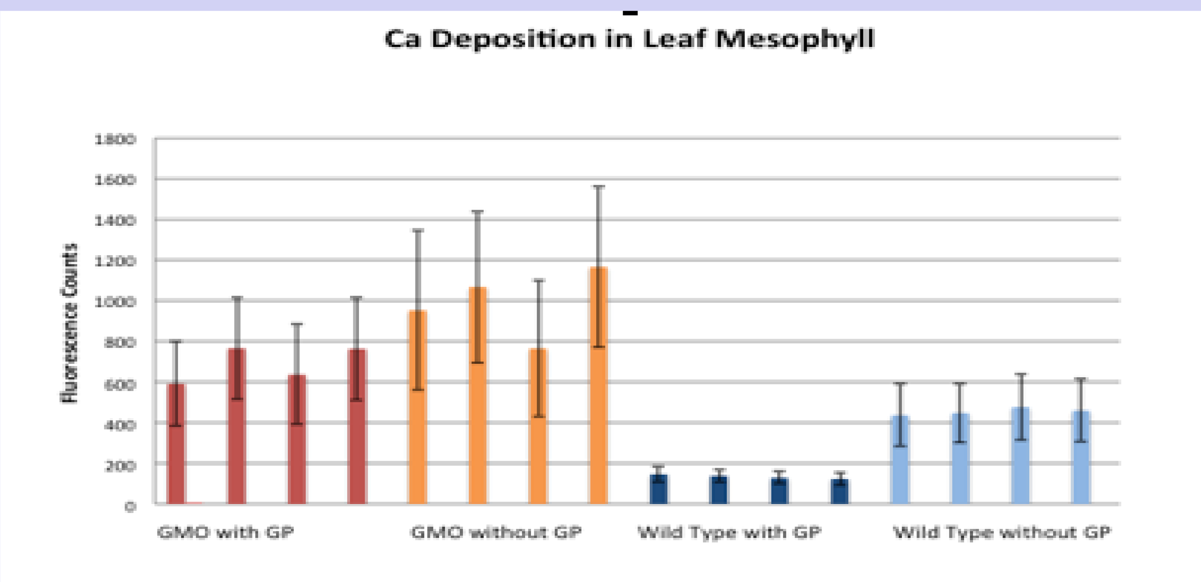


Figure 10: Graph of comparative calcium concentration in leaf mesophyll

CONCLUSIONS

Glyphosate is known to inhibit the production of essential amino acids, possibly interfering with photosynthesis and immune responses needed for healthy growth, resulting in death of plants. The GMO soybean has a genetic modification that confers resistance to the effects of glyphosate. The genetic modification did not appear to have any influence on our measured growth. We expected the WT soybean treated with glyphosate to have lesser growth and die because WT plants have no resistance to glyphosate. However, the final growth of our glyphosate-treated WT plants was not less than non-treated plants because measurements were taken before the plants experienced the full physiologic effects of glyphosate. Glyphosate has been linked to chlorosis in leaves and is a known chelator of bivalent metals. There was a decreased level of calcium in leaf mesophyll of both GMO and WT soybeans. We hypothesize that calcium translocation may be disturbed within the soybean plant. Since the glyphosate-treated WT soybean had a greater reduction in calcium levels, it is possible that the genetic modification of the GMO soybean may provide alternative mechanisms or pathways for transport, allowing for increased deposition of calcium within mesophyll tissue. Therefore, the genetic modification may affect the homeostasis of calcium within the soybean. The observed localized areas of concentrated calcium may be calcium oxalate crystals. Calcium oxalate crystals are known to accumulate in soybean tissues and are considered part of the natural metal homeostasis of soybean plant and seed.

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